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RESPONSE OF INVERTEBRATES TO GLYPHOSATE-INDUCED HABITAT ALTERATIONS IN WETLANDS

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Abstract: Wetlands in the Prairie Pothole Region of eastern North Dakota, USA are often overgrown with cattails (*Typha* spp), providing habitat for crop-depredating blackbirds and impeding use by waterfowl. One and two years post-treatment (1992 and 1993), we assessed the response of invertebrates to a catastrophic reduction in cattail coverage caused by glyphosate, a herbicide applied to about 14,000 ha of North Dakota's wetlands since 1991. Numbers of Crustacea, Hydracarina, Oligochaeta, Copepoda, Ostracoda, and Cladocera were similar between treated and reference wetlands ($P > 0.10$), while abundance of Gastropoda was greater in the treated wetlands ($P = 0.10$). Insect abundance was greater in treated wetlands ($P < 0.01$), with activity traps yielding highest numbers in July. Corixidae and Chironomidae were more abundant in treated wetlands ($P < 0.10$), whereas Chaoboridae was consistently more plentiful in the reference wetlands ($P = 0.05$). Our results suggest that populations of some aquatic invertebrates may be enhanced by a reduction in cattail coverage with glyphosate-based herbicide.

Key Words: aquatic insects, cattails, ducks, glyphosate, habitat management, invertebrates, waterfowl, wetlands

INTRODUCTION

Historically, wetland vegetation in North Dakota, USA consisted of sparse stands of bulrush (*Scirpus* spp.) and common cattail (*Typha latifolia*) (Kantrud 1986). By the 1970s, narrow-leaved cattail (*T. angustifolia* L.), a species not recorded in North Dakota until 1942, had spread throughout the state (Kantrud 1986). These native and exotic cattails hybridized to produce *T.* × *glauca* Godron, a robust plant that quickly forms dense homogenous stands (Weller 1975, Davis and van der Valk 1978). Cattail-dominated wetlands deter use by waterfowl (Kantrud 1986, Linz et al. 1996) and attract migrating blackbirds, which may cause significant amounts of crop damage (Leitch et al. 1997). Wetland managers have controlled cattails by manipulating water levels, regulating muskrat (*Ondatra zibethica*) populations, grazing with livestock, burning, mowing, discing, excavating, and blasting with explosives (Kantrud 1986).

In 1991, wildlife agencies began operational spraying of cattail-dominated wetlands in North Dakota with glyphosate-based herbicide (Homan et al. 1998). Good management practices dictate that herbicides must not have detrimental effects on aquatic invertebrate populations, a critical food source for migrating and nesting wetland-dependent birds. Glyphosate, a nonselective, nonresidual, postemergent aquatic herbicide inhibits protein synthesis by blocking the shikimic acid pathway (Ware 1989), which is absent in animals, rendering glyphosate practically nontoxic to aquatic invertebrates (Folmar et al. 1979, Buhl and Faerber 1989, Henry et al. 1994). Any potential negative effects from glyphosate treatments are probably related to the introduction of large quantities of decaying organic material (Solberg and Higgins 1993).

Solberg and Higgins (1993) assessed the post-treatment effects of aerially-applied glyphosate on overall invertebrate numbers in South Dakota wetlands. They trapped invertebrates for two consecutive days during two periods (1 May to 1 June and 10 June to 10 July 1986 and 1987) in four glyphosate-treated, four cattail-dominated reference, and four open-water reference wetlands (≥25% open water). In this paper, we augment their data by sampling invertebrates in glyphosate-treated and reference wetlands from April through August 1992 and 1993 (one and two years post-treatment).

STUDY AREA AND METHODS

Study Area

Our experimental wetlands were in the Drift Plains of eastern North Dakota in Dickey, Nelson, Sargent, Ransom, and Walsh counties. Row crops, small grains, and hay predominate this intensively farmed landscape. The area is subject to large annual variations in water coverage. From April through August, precipitation in eastern North Dakota was 37.8 cm and 54.4 cm in 1992 and 1993, respectively (North Dakota Agricultural Statistics Service 1994).

The main criteria for wetland selection were the presence of persistent water and abundant cattails. We randomly designated six wetlands to be aerially-treated with 5.8 l/ha glyphosate (Rodeo®, Monsanto Company, St. Louis, Missouri). Four wetlands served as references. The herbicide was mixed in an aqueous solution containing surfactant (Valent® X-77 Spreader, Valent USA Corporation, Greeley, Colorado), drift retardant (Chem-trol, Loveland Industries, Greeley, Colorado), and water. A fixed-wing agricultural spray plane was used to apply 15-m-wide parallel strips of herbicide along the long axes of the wetlands. About 6 m of live cattails was skipped between sprayed strips to achieve about a 70% herbicide coverage.

One and two years post-treatment (1992 and 1993), 35-mm, color-infrared aerial photographs were taken of each wetland. We identified open water and dead and live vegetation on digitally scanned 35 mm images by using color differences. Wetland size and percent coverage of water and vegetation were estimated with geospatial processing software (MicroImages, Inc., Lincoln, NE). The open water category included both open water and water covered with duckweed (*Lemna* spp.).

Invertebrate Sampling

Each wetland had three transects, with two randomly-selected sampling stations along each transect (placed without regard to habitat type). We established the first transect by finding the center of the wetland and randomly selecting a compass bearing; the remaining two transects were placed by adding 120° and 240°, respectively. We placed one 2-L clear, plastic-bottle activity trap at each sampling station for 24 h monthly from April through August in 1992 and 1993. Traps were positioned vertically in water that was at least 15 cm deep. All wetlands were sampled on approximately the same dates within a given sampling period.

Activity traps were chosen because of their cost-effectiveness and efficiency in collecting the widest range of aquatic invertebrates (Solberg and Higgins 1993). Rubber stoppers were placed in the necks of the activity traps before collecting the invertebrates. The contents were then poured through a 250-μm mesh and preserved in a 10% formalin solution for later examination. The traps were rinsed to ensure that all invertebrates were collected. Trapping ended after water depths decreased below 15 cm.

In the laboratory, invertebrates were counted and identified to family using Pennak (1978, 1989). When many microinvertebrates were present, they were subsampled before being counted with a 20× compound microscope. Subsamples were taken after diluting the sample to a known volume and then removing a known percentage of the dilution. The entire sample was scanned for rare or unusual taxa before a subsample was taken.

Water Parameters

We recorded water depth, temperature, conductivity, and pH at the same stations and on the same schedule as used for checking activity traps. Conductivity, pH, and temperature were measured with meters within five cm of the water surface and five cm of the bottom substrate at each station.

To measure dissolved oxygen (D.O.) (1993 only), soluble reactive phosphorous, and nitrate nitrogen, water samples were collected using a small hand-operated pump at one randomly-selected station along each transect. Samples were taken within the first five cm of the water surface and within five cm of the bottom substrate immediately upon arrival at the sampling station to avoid contamination by resuspended sediments. A standard 300-ml biological-oxygen-demand bottle was overflowed at least twice before collecting the D.O. sample. Additional water samples were fixed on site with Winkler reagents, and D.O. concentrations were then determined using the Winkler titration (Wetzel and Likens 1991). The water samples were analyzed for soluble reactive phosphorous (PO_4) (Watanabe and Olsen 1965) and nitrate nitrogen (NO_3-N)

Table 1. Comparison of habitat and water quality parameters between glyphosate-treated (n=6) and reference wetlands (n=4) in eastern North Dakota one and two years (averaged) post-treatment (April through August 1992 and 1993).

Parameter	Treated $\bar{x}$	Treated SE	Reference $\bar{x}$	Reference SE	F[1] Value	DF	P Value
Area (ha)	18.0	4.77	15.8	7.93	0.01	1, 4	0.93
Percent Open Water	32.7	4.63	9.8	1.59	11.02	1, 4	<0.01
Percent Live Cattail	32.7	4.93	75.1	3.88	37.38	1, 4	<0.01
Percent Dead Cattail	34.7	6.53	15.1	3.76	9.83	1, 4	0.01
Depth (cm)	53.2	7.86	55.4	6.64	0.01	1, 21	0.92
Temperature (C°)	18.1	1.14	17.6	1.84	2.08	1, 29	0.19
Dissolved O_2 (mg/l)	2.6	0.62	3.6	1.25	2.59	1, 14	0.16
PO_4 (mg/l)	0.4	0.14	0.3	0.14	0.06	1, 18	0.81
NO_3-N(mg/l)	<0.1	0.06	0.1	0.00	0.00	1, 18	0.97
Total Conduct. (uS/cm)	1,272.7	257.01	1,385.3	141.36	0.31	1, 29	0.59

[1] Repeated measures analysis of variance.

(Vendrell and Zupancic 1990) at North Dakota State University's soil testing laboratory.

Statistical Analyses

Two-factor repeated measures analyses of variances (RMANOVA) were used to examine the null hypotheses of no differences in percent coverage of open water, live cattails, and dead cattails between treated and reference wetlands across years (Cody and Smith 1991). Initially, we used one-factor analysis of variance (ANOVA) to detect differences in abundance of selected invertebrate taxa between years. We used two-factor RMANOVAs to examine the null hypotheses that (1) mean abundance of various invertebrate taxa and (2) selected water-quality variables were equal between treatments and among sampling dates (months). If a significant difference among sampling dates was detected, we used specific contrasts within the RMANOVA to identify the dates responsible. We conducted statistical analyses only on invertebrate taxa that occurred in ≥20% of the samples and had at least a mean abundance of one in the treated or reference wetlands. Count data for each taxon were normalized by square-root transformation (Montgomery 1991). We set the significance level at α = 0.1 for all statistical tests (Linz et al. 1996).

RESULTS

Habitat Characteristics

Mean wetland size was similar between treated and reference wetlands ($\bar{x}$ = 17.1 ± 4.1 ha) in August 1992 and 1993 (P = 0.93). During both years, coverages of open water and dead cattails were greater in the treated wetlands than in the reference wetlands (P < 0.01), whereas percent coverage of live cattails was greater (P < 0.01) in reference wetlands (Table 1).

Treatment effects were not detected (P > 0.18) for water temperature, depth-of-water ($\bar{x}$ = 54.3 ± 5.14 cm), pH (median = 7.4, range = 6.3–7.9), total conductivity ($\bar{x}$ = 1326.3 ± 156.08 uS/cm), PO_4 ($\bar{x}$ = 0.33 ± 0.10 mg/l), and NO_3-N ($\bar{x}$ = 0.10 ± 0.05 mg/l). Water temperatures were greater in July and August than in the previous three months, whereas pH was greater in May and July, and D.O. was lowest in August.

Invertebrate Numbers

Numbers of Copepoda, Ostracoda, and Cladocera (Chydoridae, *Daphnia* spp., *Simocephalus* spp., and *Ceriodaphnia* combined) did not differ (P > 0.23) between treated and reference wetlands (Table 2). However, Copepoda were more numerous (F = 3.77; 1, 42 df; P = 0.06) one year after treatment ($\bar{x}$ = 870.8 ± 384.43) than two years post-treatment ($\bar{x}$ = 189.32 ± 63.47) (Figures 1a and 2a). Ostracoda abundance differed among months (F = 2.70; 4, 30 df; P = 0.04), being significantly greater in July than in April, May, June, and August.

Abundances of Crustacea, Hydracarina, and Oligochaeta were similar (P > 0.12) between treated and reference wetlands, between post-treatment years (F ≤ 1.72; 1, 42 df; P ≥ 0.20), and over months (F ≤ 1.39; 4, 30 df; P ≥ 0.26) (Figures 1b and 2b). Numbers of Gastropoda were higher in the treated wetlands (P = 0.10) but were similar between years (F = 1.72; 1, 42 df; P = 0.20) and among months (F = 1.04; 4, 30 df; P = 0.40)

Overall, insect abundance was similar between post-treatment years (F = 0.32; 1, 42 df; P = 0.58) but did differ between treatments (F = 6.85; 1, 30 df; P =

Table 2. Numbers of dominant invertebrates captured in 24-h activity traps placed in glyphosate-treated (n=6) and reference wetlands (n=4) in eastern North Dakota one and two years (averaged) post-treatment (April through August 1992 and 1993).

Taxon	Treated $\bar{x}$	Treated SE	Reference $\bar{x}$	Reference SE	F[1] Value	P Value
Gastropoda	2.2	1.53	0.5	0.26	3.32	0.10
Oligochaeta	1.1	0.92	0.6	0.44	0.44	0.53
Crustacea	15.1	21.93	0.1	0.12	3.09	0.12
Copepoda	667.8	382.84	535.3	555.26	1.48	0.26
Cladocera	2,896.1	2,304.16	1,187.8	901.66	1.65	0.23
Daphnia spp.	953.2	716.72	403.4	434.29	2.39	0.16
Chydoridae	377.3	372.88	430.0	413.26	0.12	0.74
Simocephalus spp.	320.0	312.25	237.3	157.58	0.31	0.59
Ceriodaphnia spp.	1,245.6	2,009.74	117.1	110.69	1.07	0.33
Ostracoda	47.4	51.39	18.5	8.70	0.78	0.40
Hydracarina	14.4	9.63	3.0	2.76	0.02	0.88
Hemiptera	15.6	8.16	3.2	3.05	11.85	<0.01
Corixidae (A,N)[2]	15.0	8.00	3.1	3.02	9.51	0.02
Coleoptera	1.9	0.68	1.1	0.62	0.24	0.64
Dytiscidae (A,L)[3]	1.7	0.62	1.1	0.61	0.13	0.73
Diptera	23.2	5.54	18.2	7.44	1.22	0.30
Chironomidae (L)	15.0	10.68	2.3	0.24	4.50	0.07
Chaoboridae (L)	4.6	3.10	14.6	6.56	6.57	0.03
Ceratopogonidae (L)	1.3	0.64	0.7	0.39	0.67	0.44

[1] Repeated measures analysis of variance; DF = 1,30.
[2] Adults and nymphs.
[3] Adults and larvae.

0.03) and among months (F = 8.16; 4, 30 df; $P <$ 0.01), with traps yielding highest numbers in July (Figures 1c and 2c). Numbers of Coleoptera and its constituent family, Dytiscidae, were similar between post-treatment years (F $\leq$ 0.72; 1, 42 df; $P >$ 0.40) and treatments ($P \geq$ 0.64) but differed (F $\geq$ 6.92; 4, 30 df; $P \leq$ 0.01) among months. Hemiptera and member family Corixidae were similar between post-treatment years (F $\leq$ 0.86; 1, 42 df; $P \geq$ 0.36) but differed between treatments (P $\leq$ 0.01) and among months (F $\geq$ 10.36; 4, 30 df; $P \leq$ 0.01).

Abundance of Diptera differed between one ($\bar{x}$ = 15.7 $\pm$ 6.43) and two ($\bar{x}$ = 28.1 $\pm$ 7.08) years post-treatment (F = 5.01; 1, 42 df; P = 0.03) but was similar between treatments (P = 0.30) and among months (F = 1.81; 4, 30 df; P = 0.15). Chironomidae larvae numbers did not differ between years (F = 0.02; 1, 42 df; P = 0.89) but were more abundant in treated wetlands (P = 0.08), with their numbers peaking in June. In comparison, Chaoboridae larvae numbers differed between years (F = 4.23; 1, 42 df; P = 0.04) and were consistently more abundant in reference wetlands (P = 0.05), with their numbers peaking in July. Ceratopogonidae larvae numbers differed (F = 7.93; 1, 42 df; $P <$ 0.01) between the first ($\bar{x}$ = 0.65 $\pm$ 0.25) and second ($\bar{x}$ = 1.63 $\pm$ 0.52) post-treatment years and among months (F = 2.99; 4, 30 df; P = 0.03) but not between treatments (P = 0.44).

DISCUSSION

We speculated (*a priori*) that decomposition of large amounts of cattails could exert a heavy demand on D.O. while simultaneously releasing nutrients that could support large algal blooms and contaminate ground water. However, we did not detect suppressed D.O. concentrations in our treated wetlands nor did we find significantly elevated levels of NO_3-N and PO_4. Wind-driven waves and spray in the newly-formed open areas of treated wetlands may have increased the absorptive surface at the air-water interface, moving D.O. down the water column (Cole 1983) and offsetting any reduction in D.O. due to decomposition. We observed large mats of duckweed after treatment that were not present in reference wetlands, suggesting that duckweed probably benefitted from the nutrients released from the decaying cattails and from increased sunlight previously absorbed by the dense cattail canopy. Moreover, some of these nutrients may have been retained within the cattail litter by colonizing microbial populations (Murkin et al. 1989).

Within the dipterans, chironomid larvae, consisting

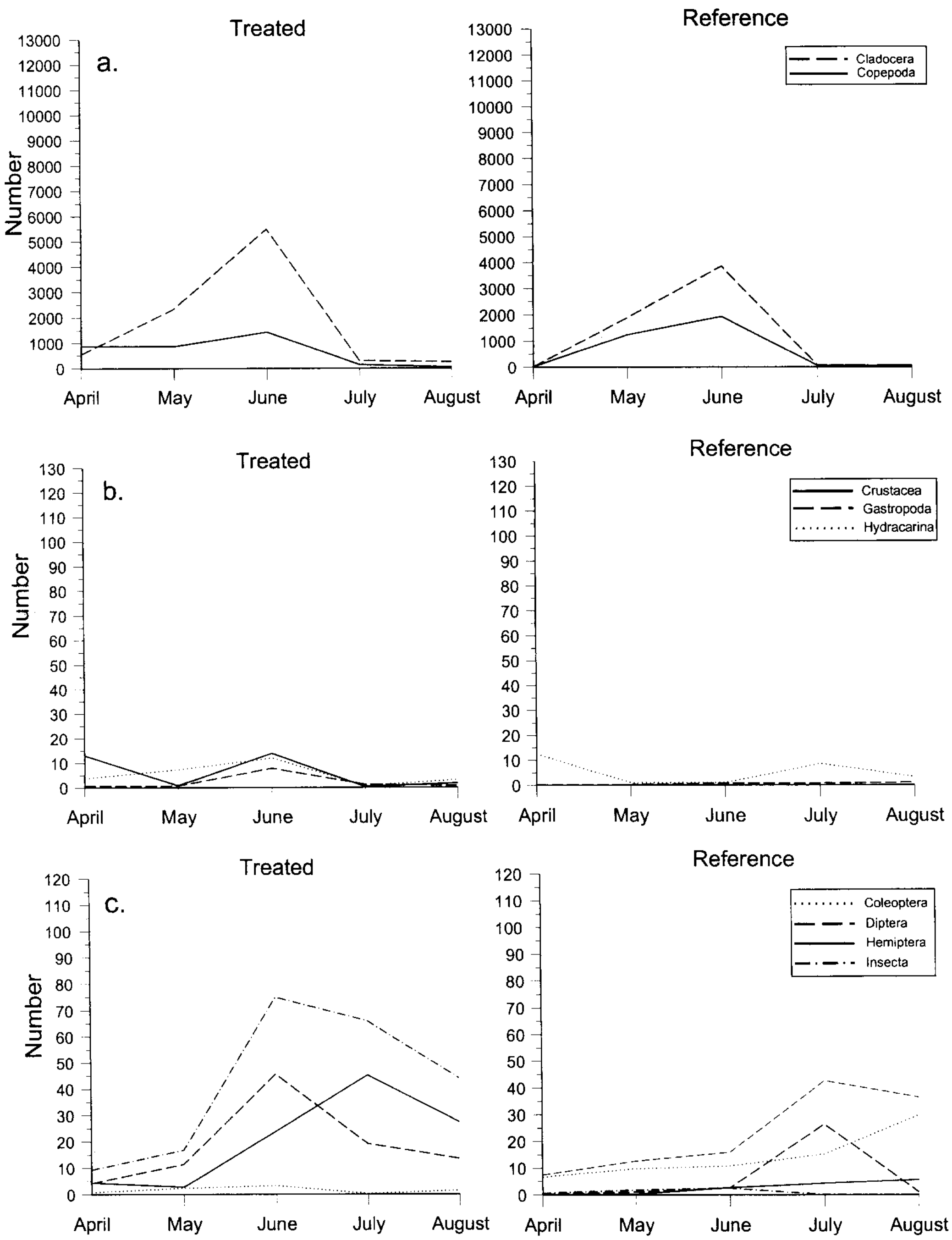

Figure 1. Mean number of dominant invertebrates captured in 24-h activity traps in glyphosate-treated (n=6) and reference (n=4) wetlands in eastern North Dakota one year post-treatment (April through August 1992).

of both nonpredaceous and predaceous species, were significantly more abundant in treated wetlands. This complex of chironomid populations may have responded to the increased availability of food resulting from decaying emergent vegetation and the increased algal production due to more sunlight at the water surface (Murkin and Kadlec 1986, Thorpe and Covich 1991). The more numerous predaceous hemipterans (largely corixids) within treated wetlands lend support to the notion that abundant prey, such as chironomids, were available after treatment. Moreover, the standing litter may have provided additional concealed perches and shelter for predators that stalk or ambush prey (Murkin et al. 1991). On the other hand, chaoborid larvae, for unknown reasons, were consistently more numerous in reference wetlands. It is unlikely that glyphosate was more toxic to chaoborids than to other aquatic invertebrates based on toxicity tests in labo-

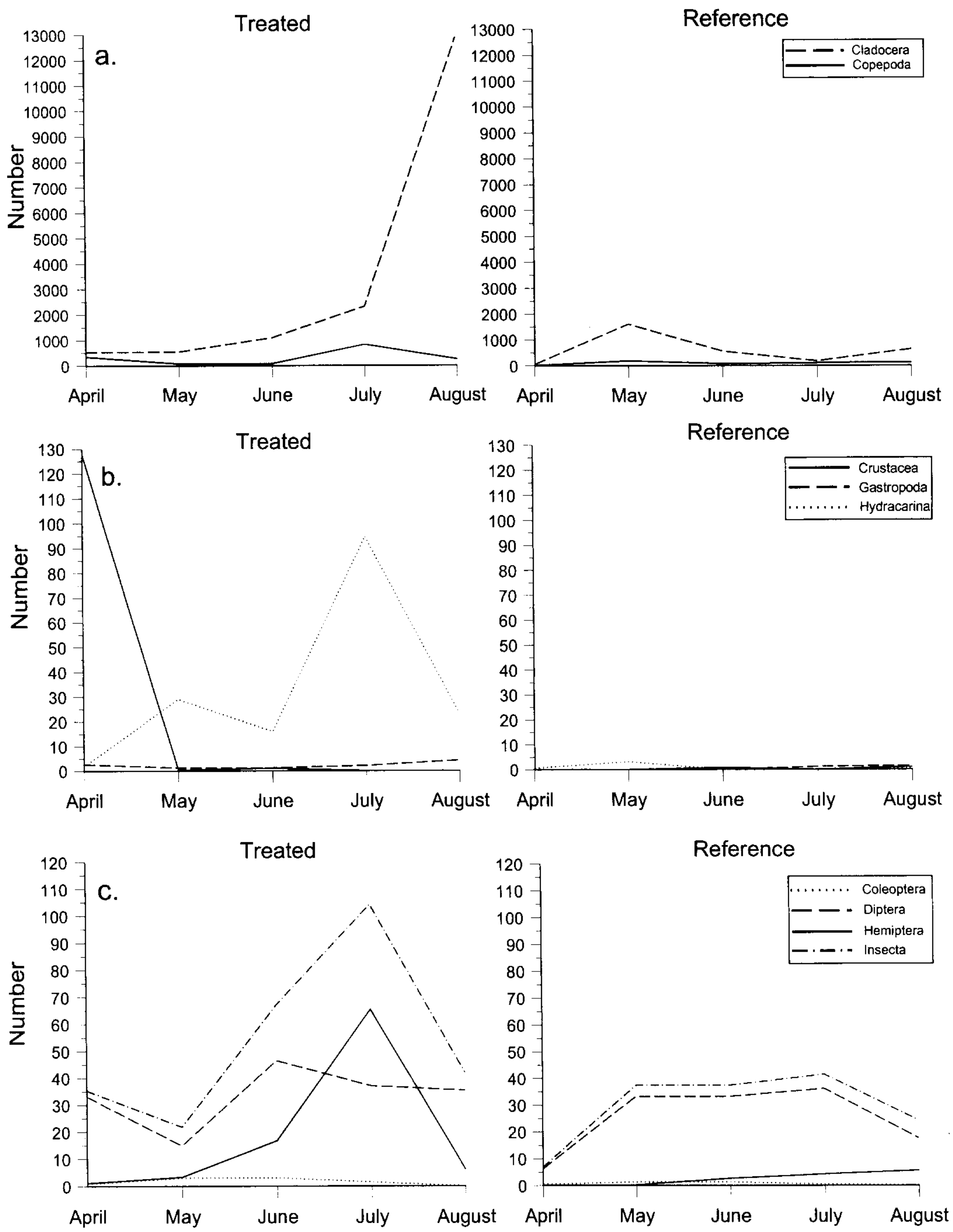

Figure 2. Mean number of dominant invertebrates captured in 24-h activity traps in glyphosate-treated (n=6) and reference (n=4) wetlands in eastern North Dakota two years post-treatment (April through August 1993).

ratory studies and field studies (Folmar et al. 1979, Buhl and Faerber 1989, Henry et al. 1994). Perhaps, changes in vegetation composition or structure are detrimental to chaoborids during certain phases of their life cycle.

Most invertebrate populations were numerically, but not statistically, more abundant in treated wetlands than in reference wetlands. High variability in the number of invertebrates within treatments suggests that other environmental factors besides coverage of water and emergent vegetation affect invertebrate populations. The size and depth of the wetland, the number of open pools, and the structure of wetland vegetation may all influence invertebrate abundance (Voights 1976, Murkin et al. 1991, Murkin et al. 1992). Under the conditions of our experiment, glyphosate treatments did not negatively affect aquatic invertebrate populations or water quality and might, in fact, have enhanced certain invertebrate populations (e.g., chironomids, hemipterans). Numerous studies have demonstrated that aquatic invertebrates within a wetland may be critical in determining use by waterfowl (Voights 1976, Kaminski and Prince 1981a and 1981b, Murkin and Kadlec 1986). Further, increased waterfowl numbers have been reported in glyphosate-treated wetlands in South Dakota (Higgins and Solberg 1993) and North Dakota (Linz et al. 1996). These birds, particularly dabbling ducks and ducklings, may have exploited the flourishing populations of invertebrates that resulted from opening the wetlands (Krapu and Swanson 1975).

Glyphosate is a non-persistent herbicide; thus, treatment effects are reversible because cattail regeneration will occur over six to ten years (G. M. Linz, person. obs.). If treatment with glyphosate is necessary, we recommend that managers stagger treatments within and among wetlands so that successional stages of emergent vegetation are present. The resultant complexity in vegetation composition and structure may lead to increased biodiversity within these wetlands. After treatment, wetland managers may also elect to plant additional foods that can successfully compete with cattail to attract wetland-dependent wildlife.

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